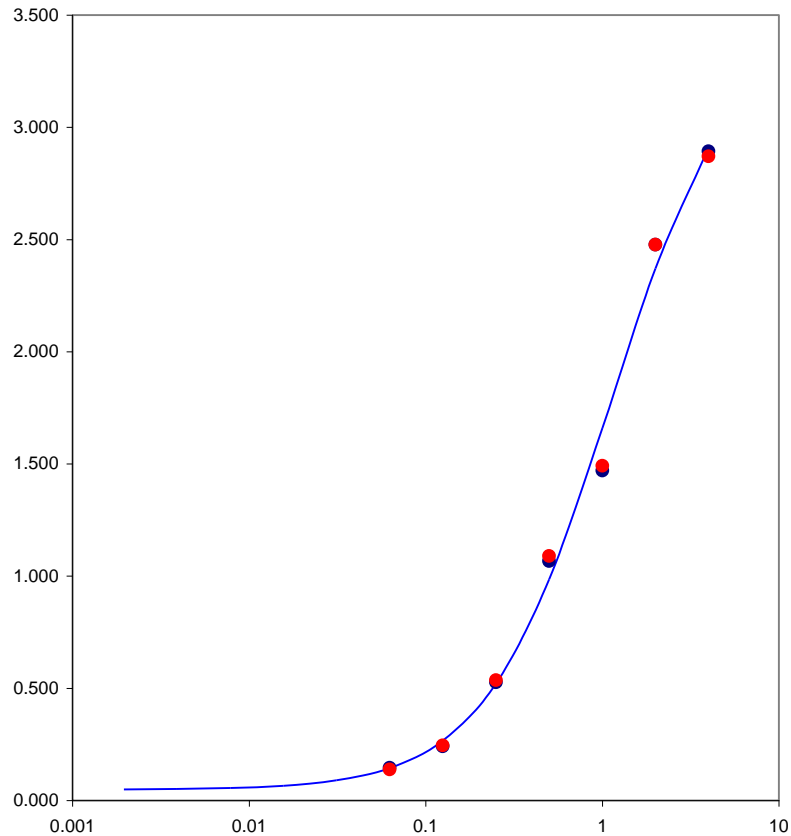


Auditable Data Analysis and Management System for ELISA (ADAMSEL-v1.1)

Conversion of OD into concentrations by a Four-Parameter Logistic Curve Fit



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Introduction

ADAMSEL FPL is an application that converts OD readings obtained from ELISA plate readers into concentrations by four-parameter fitting and ADAMSEL Merge is an application to merge results generated by ADAMSEL FPL. They are designed to provide an auditable system that minimizes data handling, thereby reducing the chances for error.

The equation used is:

$$OD = \frac{MaxOD - MinOD}{1 + e^{\frac{\ln Xmid - \ln Conc}{Scale}}} + MinOD$$

where:

<i>MaxOD</i>	=	Upper asymptote for OD
<i>MinOD</i>	=	Lower asymptote for OD (blank value)
<i>Conc</i>	=	The concentration (or reciprocal dilution)
<i>Xmid</i>	=	The midpoint of the sigmoid curve
<i>Scale</i>	=	The slope of the regression line

The application fits the sigmoid curve in two steps:

1. The starting values for MaxOD, Xmid and Scale are estimated by linear regression on logit-transformed data, where the R² is optimized by changing the MaxOD value. The starting values for Scale and Xmid are then estimated from the linear regression. The MinOD is set at the blank value observed in the data.
2. The fit is finalised by iterations of MaxOD, Xmid and Scale; where the Mean Square is minimized by changing MaxOD, Xmid and Scale.

Once the regression parameters are known, the unknown samples can be calculated with the following equation:

$$Conc = Xmid - Scale \ln \left(\frac{MaxOD - MinOD}{OD - MinOD} - 1 \right)$$

How to use ADAMSEL

Three steps are required to convert OD values into concentrations:

1. Definition of the locations and dilutions of standards and blanks and subsequent reading of the OD data. This is all done with the buttons on the Raw Data sheet.
2. Fitting the regression curve. The OD data used for the regression can be optimised by exclusion of outlying data points.
3. Saving calculated data to a file.

Disclaimer

ADAMSEL is free software for non-commercial users and comes with **ABSOLUTELY NO WARRANTY**. Please do not redistribute the application, instead refer potential users to the author of the application (remarque@bprc.nl) as this enables the distribution and maintenance of updated versions for all users.

General conventions in the application

Areas with a light green background color can be changed by the user. All other regions on the worksheet are deliberately locked and **cannot** be changed by the user. Do not attempt to paste data into the application as this is blocked to avoid the possibility of introduced error. The current application will only work correctly under Windows™ (MAC OS is not yet supported). **Set the decimal separator to a point “.”**. Users employing “Continental” settings (Dutch, German, French etc.) need to change the international settings accordingly in the Windows™ control panel. **Macro security settings in excel need to be set to medium** (Tools -> Macro -> Medium), else the application can not be used.

Define locations, dilutions and read the OD data

Figure 1. The “Raw Data” worksheet.

Plate Layout Eight_Plate100.fmt

	1	2	3	4	5	6	7	8	9	10	11	12
A	S_01Aa	S_01Ba	S_01Ca	S_01Da	S_01Ea	StdA01	StdB01	S_01Ab	S_01Bb	S_01Cb	S_01Db	S_01Eb
B	S_02Aa	S_02Ba	S_02Ca	S_02Da	S_02Ea	StdA02	StdB02	S_02Ab	S_02Bb	S_02Cb	S_02Db	S_02Eb
C	S_03Aa	S_03Ba	S_03Ca	S_03Da	S_03Ea	StdA03	StdB03	S_03Ab	S_03Bb	S_03Cb	S_03Db	S_03Eb
D	S_04Aa	S_04Ba	S_04Ca	S_04Da	S_04Ea	StdA04	StdB04	S_04Ab	S_04Bb	S_04Cb	S_04Db	S_04Eb
E	S_05Aa	S_05Ba	S_05Ca	S_05Da	S_05Ea	StdA05	StdB05	S_05Ab	S_05Bb	S_05Cb	S_05Db	S_05Eb
F	S_06Aa	S_06Ba	S_06Ca	S_06Da	S_06Ea	StdA06	StdB06	S_06Ab	S_06Bb	S_06Cb	S_06Db	S_06Eb
G	S_07Aa	S_07Ba	S_07Ca	S_07Da	S_07Ea	StdA07	StdB07	S_07Ab	S_07Bb	S_07Cb	S_07Db	S_07Eb
H	S_08Aa	S_08Ba	S_08Ca	S_08Da	S_08Ea	Blank01	Blank02	S_08Ab	S_08Bb	S_08Cb	S_08Db	S_08Eb

Format	Define	Clear
Save	Standard	Standard
Open	Sample	Samples
Clear	Blank	Blanks

Dilutions

	1	2	3	4	5	6	7	8	9	10	11	12
A	100	200	400	800	1600	1	1	100	200	400	800	1600
B	100	200	400	800	1600	2	2	100	200	400	800	1600
C	100	200	400	800	1600	4	4	100	200	400	800	1600
D	100	200	400	800	1600	8	8	100	200	400	800	1600
E	100	200	400	800	1600	16	16	100	200	400	800	1600
F	100	200	400	800	1600	32	32	100	200	400	800	1600
G	100	200	400	800	1600	64	64	100	200	400	800	1600
H	100	200	400	800	1600	1	1	100	200	400	800	1600

File Open a1cig0lj02052005012.txt

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.126	0.095	0.068	0.061	0.058	2.586	2.765	0.144	0.094	0.064	0.055	0.048
B	0.154	0.091	0.069	0.057	0.054	2.347	2.327	0.156	0.093	0.067	0.062	0.049
C	0.137	0.090	0.065	0.055	0.050	1.636	1.574	0.152	0.093	0.068	0.054	0.049
D	0.140	0.088	0.064	0.055	0.054	0.770	0.908	0.130	0.087	0.065	0.054	0.048
E	0.217	0.134	0.095	0.060	0.058	0.439	0.431	0.235	0.128	0.082	0.060	0.053
F	0.261	0.135	0.086	0.064	0.057	0.231	0.222	0.223	0.141	0.087	0.061	0.051
G	0.240	0.153	0.142	0.103	0.071	0.150	0.162	0.244	0.154	0.126	0.092	0.060
H	0.284	0.163	0.101	0.075	0.067	0.060	0.058	0.251	0.149	0.088	0.062	0.053

Od Values
Open
Clear
Names

Define the plate layout

Before reading OD data, the plate layout must be defined. The application then uses this layout to transport standards and blanks to the “Regression” worksheet. The location of standards, blanks and samples is indicated by selecting the appropriate range in the plate layout on the “Raw Data” sheet (green background area) and subsequently pressing the

appropriate define buttons for Standard, Blank or Sample. When defining standards or samples, dialog boxes are used to specify dilutions and dilution factors. For the standard, an additional dialog box is used to specify the highest concentration. Dilutions belonging to standards and samples will automatically be displayed on the “Raw Data” sheet.

Standards are indicated by a name followed by an index letter and a number. A capital index letter (A or B) defines the standard duplicates, and the index number (two digits) defines the sequential dilution of the standards. The standard can maximally use 2 x 12 wells. For example StdA01, indicates the first dilution of the first standard replicate.

Blanks are indicated by a name and an identifier number; up to 96 blanks can be used. For example Blank01, indicates the first blank

Samples can be single or duplicate; they are indicated by S_ followed by the sample number and a capital character to indicate the order. A second lower case character (a or b) is added to indicate sample duplicates. Samples can have up to 12 wells and can be either single or duplicate. For example S01Aa and S01Ba, indicate the first and second dilution of the first replicate of sample 1, respectively.

Formats can be saved and loaded with the buttons provided. The name of the format file is displayed on the “Raw Data” sheet upon opening or saving.

Read the OD data

OD data from a text (ASCII) file can be loaded by pressing the “Open” button. A file open dialog will appear and the file can be selected. OD input files can be either tab delimited text files or comma-separated files. The name of the file opened is automatically displayed on the “Raw Data” sheet. The application will automatically convert data files containing commas as a decimal separator. Overflow and non-numeric values in the OD data file will be displayed as 9.999 after the OD data have been read. The Standard and blank data will automatically be transferred to the “Regression” worksheet.

Adding sample names

After the OD data have been read, names can be read from a text file or names can be manually entered on the “Format” sheet. Do **not** enter names before opening OD data, as names will be automatically reset to default values when an OD data file is opened.

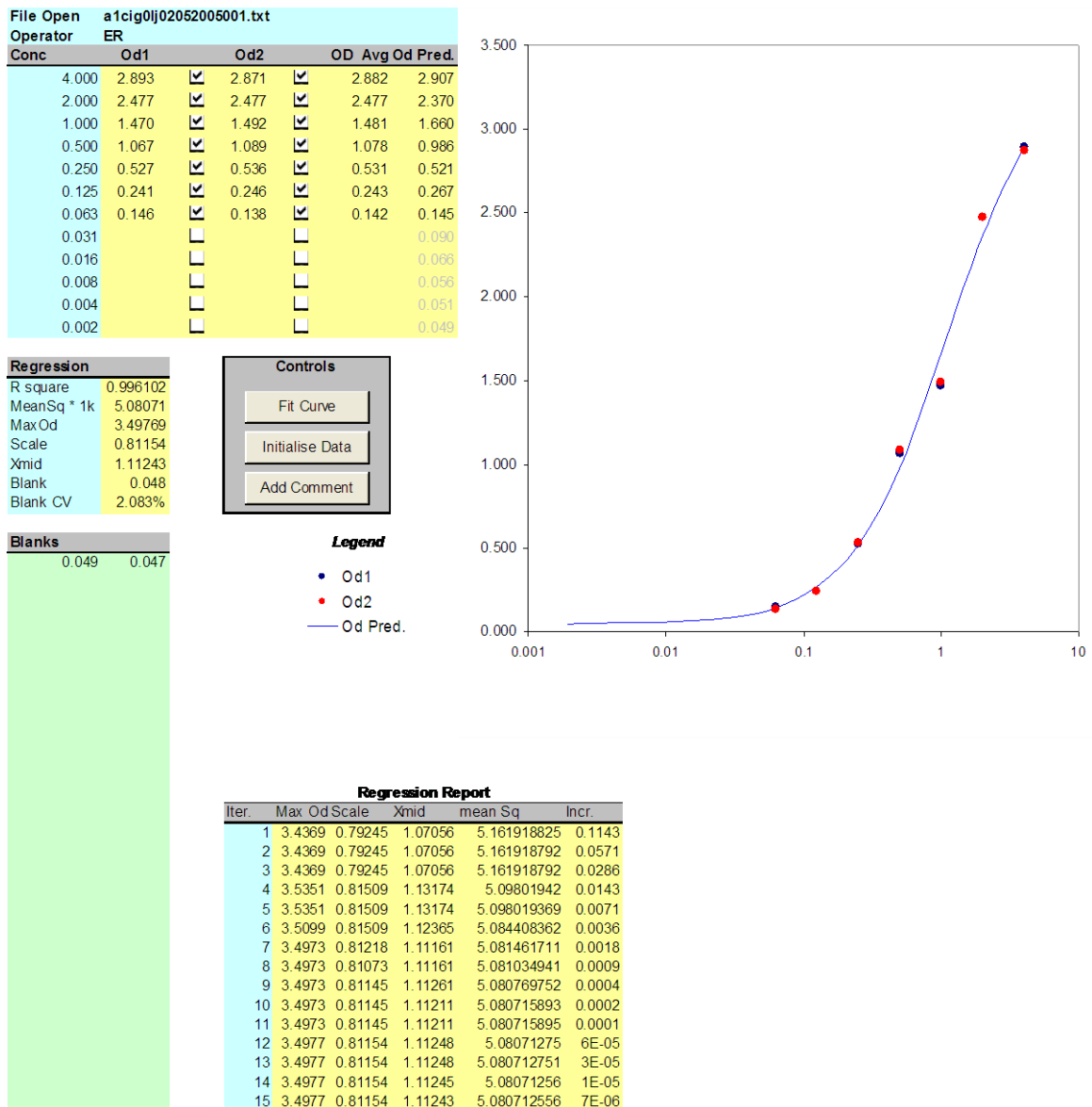
Fit the regression curve

The first fit provided on the “Regression” worksheet contains the start values for the regression, based on a linear fit on logit-transformed OD data. Data points can be excluded or included by ticking the check boxes next to the values. Overflow as well as values below the low cut-off are automatically excluded and **can not** be selected (the lower cut-off can be defined on the settings sheet). The start values will change accordingly. In a similar way, blanks returning values that are obviously out of range can also be taken out of the calculation, by deleting the outlier value. The start values will

then automatically be recalculated. The fit must be finalized by pressing the "Fit Curve" button. This starts the minimisation of the Mean Square. A report on the optimisation procedure is presented on the "Regression" worksheet. The fit can be repeated after standard points have been in- or excluded. The standard and blank values displayed on the "Regression" sheet can be reset to the start values by pressing the "Initialise Data" button. Comments can be entered, viewed and edited by pressing the "Add comment" button. Comments will be displayed on the "Results" sheet.

After the curve has been fit, the results will be automatically displayed on the "Results" sheet. When the standard changes, either by ticking check boxes or by deleting blank values, the results sheet will be cleared and the final fit must be repeated.

Figure 2. The "Regression" worksheet.



Save the results

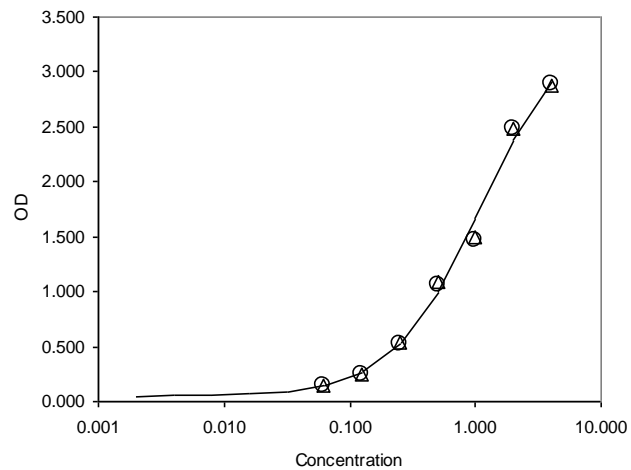
Go to the “Results” sheet and save its contents by pressing CTRL + s (saves as a text file with extension ELI) or CTRL + e (saves as a write protected Excel sheet with extension XLE). A file save dialog box will appear prompting with the filename of the OD data file. The results can be printed by pressing CTRL + p. This will print all pages of the results. Figure 3 shows the first page of the results sheets. The name of the OD data file is displayed on all pages of the results. A regression report is supplied showing the following values:

Figure 3. First page of the “Results” worksheet.

Results For a1cig0lj02052005001.txt

Regression

Operator	ER
Date	01/05/08
Rsquare	0.99610
Mean Sq x 1000	5.08071
Max OD	3.49769
Scale	0.81154
Xmid	1.11243
Blank	0.048
Blank CV (%)	2.1
High cut off OD	3.393
Low Cut off OD	0.053
Calculated [Std]	3.97553
N Std. incl.	14
Od = 1 + blank @	0.53764



Comment

Standard @	Dilution	OD1	OD2	CV OD	Conc1	Conc2	CV Conc	Incl 1	Incl 2
4	1	2.893	2.871	0.4	3.909117	3.773528	1.8	T	T
2	2	2.477	2.477	0.0	4.496542	4.496542	0.0	T	T
1	4	1.470	1.492	0.7	3.336371	3.40824	1.1	T	T
0.5	8	1.067	1.089	1.0	4.395032	4.505	1.2	T	T
0.25	16	0.527	0.536	0.8	4.047888	4.119635	0.9	T	T
0.125	32	0.241	0.246	1.0	3.59321	3.67315	1.1	T	T
0.0625	64	0.146	0.138	2.8	4.050567	3.772782	3.6	T	T

Blank	Dilution	OD1	OD2	Blank Included
1 -	1	0.049	0.047	T T

A plot showing the observed standard values (circles and triangles) as well as the predicted OD values (line) is displayed next to the regression parameters.

The standard values are tabulated under the graph. Standard @ and dilution respectively indicate the concentration and dilution of the standard in that particular well. The OD values, calculated concentrations and their corresponding CV values are displayed for each (pair) of observation(s). Incl1 and Incl2 indicate whether the well was included (T

for True) or excluded (F for False). The values for the blanks are displayed immediately below the standard values. If a blank value has been deleted on the regression sheet it will be displayed with a strikethrough as well as with an “F” under the heading blank included. Included blank values are indicated by a “T”.

<i>Field</i>	<i>Description</i>
Operator	Initials of the regression operator
Date	Date when regression was performed
Rsquare	Correlation coefficient of observed and predicted OD values. The Rsquare is calculated on log-transformed OD values
Mean Sq x 1000	The value of the mean square multiplied by 1000; defined as: $\Sigma(\text{Observed} - \text{Predicted})^2 / \text{Predicted}$ divided by the number of observations included
MaxOD	The Plateau OD value estimated by the regression procedure
Scale	A parameter indicating the steepness of the curve, where larger values indicate flatter curves.
Xmid	The midpoint of the sigmoid curve
Blank	The lower asymptote of the regression curve. This is in fact the average blank value.
Blank CV	The coefficient of variation of the blank values
High cut off OD	Concentrations for OD values above this value will be displayed as “High”
Low cut off OD	Concentrations for OD values below this value will be displayed as “Low”
Calculated [Std]	The concentration calculated for the standard using the curve fit parameters. This is the standard regressed on itself
N Std. Incl.	The number of data points included for the standard
OD = 1 + blank @	Indicates at which concentration an OD of 1 over blank will be achieved

Figure 4. Second page of the “Results” worksheet

Results For a1cig0lj02052005001.txt

Sample Name	Dilution	Od1	Od2	CV Od	Conc1	Conc2	CV Conc
sample01	100	0.440	0.455	1.7	21.00344	21.73996	1.7
sample01	200	0.247	0.239	1.6	23.05699	22.25742	1.8
sample01	400	0.144	0.125	7.1	24.88389	20.71081	9.2
sample01	800	0.086	0.076	6.2	23.13496	18.01381	12.4
sample01	1600	0.064	0.062	1.6	Low	Low	
sample02	100	0.495	0.508	1.3	23.71173	24.35547	1.3
sample02	200	0.232	0.279	9.2	21.55554	26.23277	9.8
sample02	400	0.125	0.124	0.4	20.71081	20.48733	0.5
sample02	800	0.079	0.075	2.6	19.57889	17.48578	5.6
sample02	1600	0.109	0.062	27.5	68.3113	Low	
sample03	100	0.534	0.534	0.0	25.64799	25.64799	0.0
sample03	200	0.256	0.282	4.8	23.95354	26.52898	5.1
sample03	400	0.126	0.137	4.2	20.93387	23.3614	5.5
sample03	800	0.077	0.076	0.7	18.53859	18.01381	1.4
sample03	1600	0.062	0.057	4.2	Low	Low	
sample04	100	0.583	0.577	0.5	28.10508	27.80258	0.5
sample04	200	0.284	0.270	2.5	26.72633	25.34274	2.7
sample04	400	0.134	0.136	0.7	22.70384	23.14257	1.0
sample04	800	0.083	0.080	1.8	21.6259	20.09467	3.7
sample04	1600	0.060	0.060	0.0	Low	Low	
sample05	100	1.011	0.929	4.2	51.51367	46.67897	4.9
sample05	200	0.635	0.666	2.4	61.49673	64.68861	2.5
sample05	400	0.345	0.350	0.7	65.42621	66.40415	0.7
sample05	800	0.177	0.178	0.3	63.76313	64.17966	0.3
sample05	1600	0.112	0.108	1.8	71.07642	67.38494	2.7
sample06	100	0.914	0.824	5.2	45.81594	40.76368	5.8
sample06	200	0.577	0.514	5.8	55.60515	49.30624	6.0
sample06	400	0.275	0.284	1.6	51.67493	53.45267	1.7
sample06	800	0.145	0.161	5.2	50.20024	57.04303	6.4
sample06	1600	0.095	0.087	4.4	55.09938	47.26689	7.7
sample07	100	1.072	0.935	6.8	55.24881	47.02596	8.0
sample07	200	0.707	0.719	0.8	68.96168	70.22419	0.9
sample07	400	0.378	0.392	1.8	71.87777	74.61458	1.9
sample07	800	0.192	0.190	0.5	69.97373	69.15003	0.6
sample07	1600	0.109	0.117	3.5	68.3113	75.64127	5.1
sample08	100	1.121	0.793	17.1	58.34311	39.06984	19.8
sample08	200	0.491	0.479	1.2	47.02794	45.84302	1.3
sample08	400	0.240	0.237	0.6	44.71503	44.11422	0.7
sample08	800	0.124	0.125	0.4	40.97466	41.42162	0.5
sample08	1600	0.091	0.080	6.4	51.21335	40.18934	12.1

The results for the individual wells are displayed on page 2 of the results worksheet. The sample names (if entered), dilutions, OD values and concentrations as well as the CV values are presented in a table.

Figure 5. Third page of the “Results” worksheet

Results For a1cig0lj02052005001.txt

Sample Name	Valid N	Oadmin	Odmax	Conc	CVconc	wConc	CVwc
sample01	8	0.062	0.455	21.8502	8.7	21.94616	5.4
sample02	8	0.062	0.508	21.7648	12.2	23.6666	4.8
sample03	8	0.057	0.534	22.8283	13.5	25.4405	4.2
sample04	8	0.060	0.583	24.443	11.4	27.67421	3.6
sample05	10	0.108	1.011	62.2612	11.4	56.82059	13.1
sample06	10	0.087	0.914	50.6228	9.4	48.1087	12.5
sample07	10	0.109	1.072	67.1029	12.7	61.27604	16.0
sample08	10	0.080	1.121	45.2912	12.3	48.21942	18.6

The third page of the “Results” sheet shows the aggregated data for each sample. Where a sample has multiple dilutions, the data are reduced to one value for each sample. Duplicates with one or both concentrations at Low or High are excluded from the calculations, as well as samples with a CV higher than the specified maximum CV for duplicates (Specified on the “Settings” sheet). The number of data points included as well

as the minimum and maximum OD values for the included data points are tabulated. Concentrations are displayed as the average of all included data points as well as a weighted average. Weights are assigned based on OD values (with blank subtracted), where OD values over 2.000 are assigned a weight of 1; OD values between 2.000 and 0.500 are assigned a weight of 100; OD values smaller then 0.500 and greater then 0.200 are assigned a weight of 10 and OD value lower or equal then 0.200 are assigned weight 1. This weighting is designed to make concentration estimates for unknowns more dependent on OD values within the linear range of the standard curve. The CV's for the averaged concentration as well as for the weighted concentration are also displayed. The aggregated data are saved in the results file as well as in a separate file with the same name, but with extension "uni" (for units). The "eli" and "xle" files can then later be merged to obtain a file with all titration results that enables auditing (see later under ADAMSEL Merge).

The Settings sheet

Figure 6. The "Settings" worksheet

Od Data File	
# Empty Lines in OD File	0
Overflow Value	6
File Extension for OD File	txt
File extension for Names File	naa
Cut Off values	
Cut Off Lo	0.072
Cut Off Hi	3.393
Minimum & Maximum cut off settings	
Maximum obs in Std	2.893
Tolerance High OD	0.500
Tolerance Low OD (x Blank)	1.500
CV cut off (%)	30
Regression settings	
Number of iterations	15
Number of steps per iteration	7
Application Data	
Version	β 024
Date	09/06/2007
Copyrights	EJ Remarque

The settings worksheet enables the user to change seven application settings. Only values displayed on a green background can be changed by the user.

<i>Field</i>	<i>Description</i>
# empty lines in OD file	Some plate readers add additional lines in their output before the actual OD data are given. The number of lines specified here will be skipped when reading OD data from a file.
Overflow value	OD readings greater than the value specified will be displayed as overflow (9.999) on the “Raw Data” sheet these values will be ignored by the application and a “High” value will be returned for the corresponding concentrations
File Extension for OD File	This can be used to filter the files shown in the OD file open dialog box. In order to avoid seeing too many files, specify the file extension for the OD data files in this field
File Extension for Names File	This can be used to filter the files shown in the Names file open dialog box. In order to avoid seeing too many files, specify the file extension for the Names files in this field
Tolerance High OD	The value specified here will be used for extrapolations. The value will be added to the maximum OD value observed for the standard. Concentrations for OD values above the highest standard OD observed plus the specified value will be returned as “High”
Tolerance Low OD	The value specified here will be used for the low cut off. Concentrations for OD values below blank value * tolerance OD low will be returned as “Low”
CV cut off	Duplicates with a CV value higher than the one specified here will be excluded from the calculation of the aggregated means

The Format sheet

Figure 7. The “Format” sheet

FormatName C:\Documents and Settings\Eigenaar\My Documents\Ed_I

Name	Replicates	Wells	Dilute_1	Dil_Factor	[First]
Standard	2	7	1	2	4
Blanks	1	2	1	1	
Sample01	2	5	50	2	
Sample02	2	5	100	2	
Sample03	2	5	50	2	
Sample04	2	5	100	2	
Sample05	2	5	50	2	
Sample06	2	5	100	2	
Sample07	2	5	50	2	
Sample08	2	5	100	2	

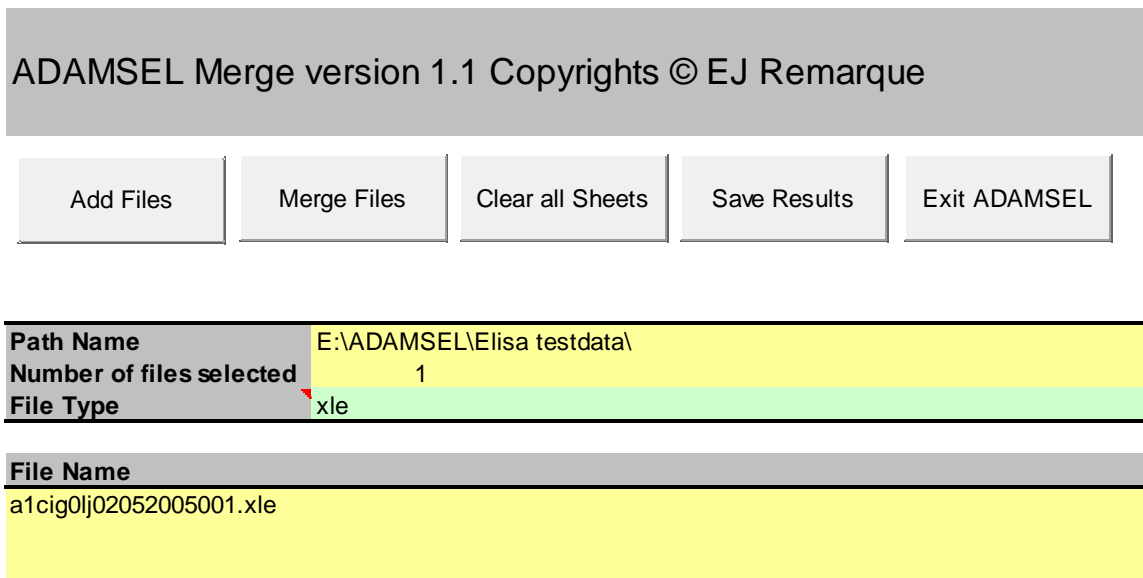
The format sheet should be used to add sample names **after** OD data have been read. Note that sample names are automatically reset to default values (Sample01 etc.) when OD data are read. The “Format” sheet also offers the opportunity to change sample dilutions and dilution factors. These are initially entered when defining a format, but can be changed later. Any change made on the “Format” sheet will be automatically

implemented. If sample dilutions change, the changes are displayed on the “Raw data” and “Results” sheets. Settings for Standards and blanks can not be changed. The standard and blank, can, however, be re-entered after they have been cleared, using the buttons on the “Raw Data” sheet.

Merging calculated results from multiple plates

Once a series of ELISA plates has been calculated, the results can be merged into one Excel workbook, allowing for easy inspection of the results from whole series. Usually only the calculated data for the samples are saved. The “ADAMSEL merge” application, however, allows for the aggregation of all results obtained with the ADAMSEL FPL application. The results obtained for Regression, Standards, Blanks, Separate Samples and Aggregated Samples will be displayed on separate worksheets.

Figure 8. Main screen of the ADAMSEL Merge application



After pressing the “Add Files” button, a dialog box will appear and the files to be merged can be selected; only files of the type indicated at File Type will be shown in the file open dialog box. Two values can be used: eli, for text results files and xle for Excel results files. After pressing the “Merge Files” button all results will be merged to 5 separate worksheets within the workbook. The data can then be saved as an Excel workbook by pressing the “Save Results” button. The Excel file thus generated will contain all the results without any Excel Macros. All information contained in the results files will be displayed on the worksheets generated. Strikethrough values on the Standard and Blank worksheets indicate that a certain value was not included in the calculations.

Figure 9. Regression worksheet of the ADAMSEL Merge application

File	Operator	Date	Requare	Mean Sq	Max Od	Scale	Xmid	Blank	Blank CV	High cut off Od	Low Cut off Od	Calculated [Std]	N Std. inc Od = 1 +	Hi Cut C	Lo Cut C	Comment
a1cig0lj02052005001.txt	ER	14/09/07	0.996102	5.080713	3.497688	0.811544	1.112435	0.048	2.083333	3.393	0.0528	3.975532322	14	0.537643	16.50288	0.005363

Figure 10. Standards worksheet of the ADAMSEL Merge application

File	Standard	Dilution	Od1	Od2	CV Od	Conc1	Conc2	CV Conc	Incl 1	Incl 2
a1cig0lj02052005001.txt	4	1	2.893	2.871	0.381679	3.909117	3.773528	1.764874	T	T
a1cig0lj02052005001.txt	2	2	2.477	2.477	0	4.496542	4.496542	0.00E+00	T	T
a1cig0lj02052005001.txt	1	4	1.47	1.492	0.742741	3.336371	3.40824	1.065587	T	T
a1cig0lj02052005001.txt	0.5	8	1.067	1.089	1.020408	4.395032	4.505	1.235598	T	T
a1cig0lj02052005001.txt	0.25	16	0.527	0.536	0.84666	4.047888	4.119635	0.878434	T	T
a1cig0lj02052005001.txt	0.125	32	0.241	0.246	1.026694	3.59321	3.67315	1.10014	T	T
a1cig0lj02052005001.txt	0.0625	64	0.146	0.138	2.816901	4.050567	3.772782	3.550709	T	T
a1cig0lj02052005003.txt	4	1	2.834	2.901	1.17E+00	3.487189	4.014359	7.03E+00	T	T
a1cig0lj02052005003.txt	2	2	2.484	2.508	0.480769	4.101578	4.225794	1.49166	T	T
a1cig0lj02052005003.txt	1	4	1.745	1.853	3.001668	3.888738	4.290463	4.911551	T	T
a1cig0lj02052005003.txt	0.5	8	0.997	1.023	1.287129	3.81428	3.922584	1.399844	T	T
a1cig0lj02052005003.txt	0.25	16	0.5	0.535	3.381643	3.853022	4.105738	3.175319	T	T
a1cig0lj02052005003.txt	0.125	32	0.245	0.26	2.970297	3.942981	4.175601	2.86528	T	T
a1cig0lj02052005003.txt	0.0625	64	0.136	0.136	0	4.246325	4.246325	0	T	T
a1cig0lj02052005004.txt	4	1	2.848	2.82	0.494001	3.728819	3.60283	1.718416	T	T
a1cig0lj02052005004.txt	2	2	2.312	2.366	1.154339	4.273554	4.490342	2.473647	T	T
a1cig0lj02052005004.txt	1	4	1.436	1.442	0.208478	4.020304	4.041738	0.265857	T	T
a1cig0lj02052005004.txt	0.5	8	0.643	0.75	7.681263	3.368368	3.920486	7.574824	T	T
a1cig0lj02052005004.txt	0.25	16	0.371	0.369	0.27027	3.989013	3.968663	0.255729	T	T
a1cig0lj02052005004.txt	0.125	32	0.185	0.212	6.801007	4.034163	4.638978	6.97343	T	T
a1cig0lj02052005004.txt	0.0625	64	0.114	0.115	0.436681	4.635897	4.68818	0.560727	T	T
a1cig0lj02052005006.txt	4	1	2.769	2.805	0.645856	3.642025	3.762946	1.632969	T	T
a1cig0lj02052005006.txt	2	2	2.172	2.383	4.632272	4.346778	5.200486	8.941914	T	T
a1cig0lj02052005006.txt	1	4	1.187	1.033	6.936937	3.480983	2.913358	8.876979	T	T
a1cig0lj02052005006.txt	0.5	8	0.808	0.8	0.497512	4.314471	4.263508	0.594113	T	T
a1cig0lj02052005006.txt	0.25	16	0.426	0.434	0.930233	4.105458	4.194117	1.068232	T	T
a1cig0lj02052005006.txt	0.125	32	0.219	0.221	0.454545	3.734783	3.777383	0.567082	T	T
a1cig0lj02052005006.txt	0.0625	64	0.143	0.149	2.054795	4.224124	4.481863	2.960484	T	T
a1cig0lj02052005007.txt	4	1	3.042	2.854	3.188602	4.603177	2.639924	27.10515	T	T
a1cig0lj02052005007.txt	2	2	4.97	4.772	5.291288	4.693032	4.412268	9.04145	F	F
a1cig0lj02052005007.txt	1	4	2.351	2.245	2.306353	4.979265	4.43896	5.736795	T	T
a1cig0lj02052005007.txt	0.5	8	1.319	1.133	7.585645	3.750016	3.135324	8.92755	T	T
a1cig0lj02052005007.txt	0.25	16	0.788	0.756	2.072539	4.277611	4.10563	2.051478	T	T
a1cig0lj02052005007.txt	0.125	32	0.353	0.366	1.808067	3.999641	4.137755	1.697271	T	T
a1cig0lj02052005007.txt	0.0625	64	0.178	0.182	1.111111	4.026236	4.126475	1.229513	T	T

Figure 11. Blanks worksheet of the ADAMSEL Merge application

File	Blank1	Blank2	Blank3	Blank4
a1cig0lj02052005001.txt	0.049	0.047		
a1cig0lj02052005003.txt	0.054	0.046		
a1cig0lj02052005004.txt	0.046	0.078		
a1cig0lj02052005006.txt	0.051	0.049		
a1cig0lj02052005007.txt	0.056	0.065		

Figure 12. Samples all worksheet of the ADAMSEL Merge application

File	Names	Sample	N	Dilution	Od1	Od2	CV Od	Conc1	Conc2	CV Conc
a1cig0lj02052005001.txt	plan 2256.	01.047.0		100	0.44	0.455	1.675978	21.00344	21.73996	1.723107
a1cig0lj02052005001.txt	plan 2256.	01.047.0		200	0.247	0.239	1.646091	23.05699	22.25742	1.764495
a1cig0lj02052005001.txt	plan 2256.	01.047.0		400	0.144	0.125	7.063197	24.88389	20.71081	9.152555
a1cig0lj02052005001.txt	plan 2256.	01.047.0		800	0.086	0.076	6.172839	23.13496	18.01381	12.44544
a1cig0lj02052005001.txt	plan 2256.	01.047.0		1600	0.064	0.062	1.587302	Low	Low	
a1cig0lj02052005001.txt	plan 2256.	01.047.2		100	0.495	0.508	1.296112	23.71173	24.35547	1.339242
a1cig0lj02052005001.txt	plan 2256.	01.047.2		200	0.232	0.279	9.197652	21.55554	26.23277	9.787383
a1cig0lj02052005001.txt	plan 2256.	01.047.2		400	0.125	0.124	0.401606	20.71081	20.48733	0.542453
a1cig0lj02052005001.txt	plan 2256.	01.047.2		800	0.079	0.075	2.597403	19.57889	17.48578	5.647187
a1cig0lj02052005001.txt	plan 2256.	01.047.2		1600	0.109	0.062	27.48538	68.3113	Low	
a1cig0lj02052005001.txt	plan 2256.	01.047.4		100	0.534	0.534	0	25.64799	25.64799	0
a1cig0lj02052005001.txt	plan 2256.	01.047.4		200	0.256	0.282	4.832714	23.95354	26.52898	5.101632
a1cig0lj02052005001.txt	plan 2256.	01.047.4		400	0.126	0.137	4.182509	20.93387	23.3614	5.480347
a1cig0lj02052005001.txt	plan 2256.	01.047.4		800	0.077	0.076	0.653595	18.53859	18.01381	1.435672
a1cig0lj02052005001.txt	plan 2256.	01.047.4		1600	0.062	0.057	4.201681	Low	Low	
a1cig0lj02052005001.txt	plan 2256.	01.047.6		100	0.583	0.577	0.517241	28.10508	27.80258	0.541079
a1cig0lj02052005001.txt	plan 2256.	01.047.6		200	0.284	0.27	2.527076	26.72633	25.34274	2.657234
a1cig0lj02052005001.txt	plan 2256.	01.047.6		400	0.134	0.136	0.740741	22.70384	23.14257	0.956957
a1cig0lj02052005001.txt	plan 2256.	01.047.6		800	0.083	0.08	1.840491	21.6259	20.09467	3.670193
a1cig0lj02052005001.txt	plan 2256.	01.047.6		1600	0.06	0.06	0	Low	Low	
a1cig0lj02052005001.txt	plan 2256.	01.055.0		100	1.011	0.929	4.226804	51.51367	46.67897	4.923684
a1cig0lj02052005001.txt	plan 2256.	01.055.0		200	0.635	0.666	2.382782	61.49673	64.68861	2.529518
a1cig0lj02052005001.txt	plan 2256.	01.055.0		400	0.345	0.35	0.719424	65.42621	66.40415	0.741821
a1cig0lj02052005001.txt	plan 2256.	01.055.0		800	0.177	0.178	0.28169	63.76313	64.17966	0.32556
a1cig0lj02052005001.txt	plan 2256.	01.055.0		1600	0.112	0.108	1.818182	71.07642	67.38494	2.666072
a1cig0lj02052005001.txt	plan 2256.	01.055.2		100	0.914	0.824	5.178366	45.81594	40.76368	5.835395
a1cig0lj02052005001.txt	plan 2256.	01.055.2		200	0.577	0.514	5.774519	55.60515	49.30624	6.004027
a1cig0lj02052005001.txt	plan 2256.	01.055.2		400	0.275	0.284	1.610018	51.67493	53.45267	1.691031
a1cig0lj02052005001.txt	plan 2256.	01.055.2		800	0.145	0.161	5.228758	50.20024	57.04303	6.380628
a1cig0lj02052005001.txt	plan 2256.	01.055.2		1600	0.095	0.087	4.395604	55.09938	47.26689	7.651439
a1cig0lj02052005001.txt	plan 2256.	01.055.4		100	1.072	0.935	6.826109	55.24881	47.02596	8.039955
a1cig0lj02052005001.txt	plan 2256.	01.055.4		200	0.707	0.719	0.841515	68.96168	70.22419	0.907071
a1cig0lj02052005001.txt	plan 2256.	01.055.4		400	0.378	0.392	1.818182	71.87777	74.61458	1.868229
a1cig0lj02052005001.txt	plan 2256.	01.055.4		800	0.192	0.19	0.52356	69.97373	69.15003	0.592059
a1cig0lj02052005001.txt	plan 2256.	01.055.4		1600	0.109	0.117	3.539823	68.3113	75.64127	5.091936
a1cig0lj02052005001.txt	plan 2256.	01.055.6		100	1.121	0.793	17.13689	58.34311	39.06984	19.78512
a1cig0lj02052005001.txt	plan 2256.	01.055.6		200	0.491	0.479	1.237113	47.02794	45.84302	1.275873
a1cig0lj02052005001.txt	plan 2256.	01.055.6		400	0.24	0.237	0.628931	44.71503	44.11422	0.676362
a1cig0lj02052005001.txt	plan 2256.	01.055.6		800	0.124	0.125	0.401606	40.97466	41.42162	0.542453
a1cig0lj02052005001.txt	plan 2256.	01.055.6		1600	0.091	0.08	6.432748	51.21335	40.18934	12.06092

Figure 13. Samples worksheet of the ADAMSEL Merge application

File	Names	Name	Valid N	Odmin	Odmax	Conc	CVconc	wConc	CVwc
a1cig0lj02052005001.txt	plan 2256.	01.047.0	8	0.062	0.455	21.85016	8.746817	21.94616	5.370849
a1cig0lj02052005001.txt	plan 2256.	01.047.2	8	0.062	0.508	21.76479	12.19292	23.6666	4.8426
a1cig0lj02052005001.txt	plan 2256.	01.047.4	8	0.057	0.534	22.82827	13.5298	25.4405	4.22731
a1cig0lj02052005001.txt	plan 2256.	01.047.6	8	0.06	0.583	24.44296	11.41504	27.67421	3.604
a1cig0lj02052005001.txt	plan 2256.	01.055.0	10	0.108	1.011	62.26125	11.37831	56.82059	13.09753
a1cig0lj02052005001.txt	plan 2256.	01.055.2	10	0.087	0.914	50.62281	9.446438	48.1087	12.45358
a1cig0lj02052005001.txt	plan 2256.	01.055.4	10	0.109	1.072	67.10293	12.66992	61.27604	16.03628
a1cig0lj02052005001.txt	plan 2256.	01.055.6	10	0.08	1.121	45.29121	12.26949	48.21942	18.61538
a1cig0lj02052005003.txt	plan 2256.	01.047.0	6	0.054	0.447	18.04095	10.40514	18.29553	12.94812
a1cig0lj02052005003.txt	plan 2256.	01.047.2	4	0.059	0.521	18.58705	8.03584	18.33825	8.601998
a1cig0lj02052005003.txt	plan 2256.	01.047.4	10	0.134	1.915	107.8949	3.181099	109.1862	3.180318
a1cig0lj02052005003.txt	plan 2256.	01.047.6	10	0.114	1.749	88.83397	5.078059	89.18075	5.317891
a1cig0lj02052005003.txt	plan 2256.	01.055.0	8	0.063	0.376	23.51015	18.23189	19.99825	15.06165
a1cig0lj02052005003.txt	plan 2256.	01.055.2	8	0.057	0.422	23.58638	12.06346	21.32436	11.20417
a1cig0lj02052005003.txt	plan 2256.	01.055.4	6	0.056	0.362	20.1249	12.46805	18.32088	10.5617
a1cig0lj02052005003.txt	plan 2256.	01.055.6	6	0.054	0.379	20.90949	13.42257	19.70537	13.04215
a1cig0lj02052005004.txt	None	sample01	6	0.056	0.418	15.6898	2.288295	15.62915	1.438487
a1cig0lj02052005004.txt	None	sample02	4	0.051	0.334	19.27347	14.01895	19.61272	14.54545
a1cig0lj02052005004.txt	None	sample03	4	0.053	0.243	15.73424	3.749794	15.38975	2.490683
a1cig0lj02052005004.txt	None	sample04	4	0.05	0.237	16.93452	8.135661	16.21853	5.33041
a1cig0lj02052005004.txt	None	sample05	6	0.052	0.259	17.63551	3.176976	17.3658	2.719539
a1cig0lj02052005004.txt	None	sample06	6	0.051	0.246	16.79409	4.374485	16.80299	2.677709
a1cig0lj02052005004.txt	None	sample07	6	0.05	0.229	16.14603	3.294093	15.84273	2.534866
a1cig0lj02052005004.txt	None	sample08	4	0.05	0.268	14.564	14.86167	13.92094	8.809413
a1cig0lj02052005006.txt	None	sample01	6	0.05	0.325	12.86278	23.13855	15.42807	19.46886
a1cig0lj02052005006.txt	None	sample02	6	0.055	0.311	12.89628	20.89757	14.85774	17.92865
a1cig0lj02052005006.txt	None	sample03	6	0.052	0.37	17.64306	16.33762	18.93225	11.20831
a1cig0lj02052005006.txt	None	sample04	4	0.051	0.16	10.21402	5.393743	10.21402	5.393743
a1cig0lj02052005006.txt	None	sample05	6	0.054	0.188	10.21998	14.67582	8.795011	16.32673
a1cig0lj02052005006.txt	None	sample06	4	0.051	0.16	7.075864	7.879111	7.075864	7.879111
a1cig0lj02052005006.txt	None	sample07	4	0.05	0.172	8.504398	7.077228	7.958026	5.932804
a1cig0lj02052005006.txt	None	sample08	6	0.052	0.351	16.52466	14.24365	17.51257	11.81382
a1cig0lj02052005007.txt	None	sample01	6	0.069	0.353	12.62601	10.24581	12.01474	10.87387
a1cig0lj02052005007.txt	None	sample02	4	0.058	0.244	10.06015	14.34857	10.36971	15.86625
a1cig0lj02052005007.txt	None	sample03	6	0.058	0.275	11.48332	20.74097	10.15377	11.65577
a1cig0lj02052005007.txt	None	sample04	6	0.061	0.298	10.68459	10.94204	10.11751	7.673429
a1cig0lj02052005007.txt	None	sample05	6	0.082	0.436	16.69441	17.20501	15.37216	9.965936
a1cig0lj02052005007.txt	None	sample06	6	0.065	0.509	18.8854	10.88283	18.35382	7.958197
a1cig0lj02052005007.txt	None	sample07	6	0.07	0.488	16.91248	7.421614	16.77414	9.544371
a1cig0lj02052005007.txt	None	sample08	6	0.064	0.419	17.54251	16.42403	17.09889	19.09645